"A STUDY OF EVALUATION OF FAINE'S CRITERIA AND IgM ELISA IN THE DIAGNOSIS OF LEPTOSPIROSIS"

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MADRAS MEDICAL COLLEGE

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CERTIFICATE

Certified that this dissertation entitled **"A STUDY OF EVALUATION OF FAINE'S CRITERIA AND IgM ELISA IN THE DIAGNOSIS OF LEPTOSPIROSIS"** is a bonafide work done by **DR.B.PARTHIBAN,** Post Graduate student of Paediatric Medicine, Institute of Child Health and Hospital for Children, Egmore, Chennai – 08. Under our direct supervision and guidance during the academic year 2009-2012.

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DECLARATION

I declare that this dissertation entitled **"A STUDY OF EVALUATION OF FAINE'S CRITERIA AND IgM ELISA IN THE DIAGNOSIS OF LEPTOSPIROSIS"** has been conducted by me at Institute of Child Health and Hospital for Children Chennai-08. It is submitted in part of fulfillment of the award of the degree of M.D., (Paediatrics) for the April 2012 examination to be held under The Tamil Nadu DR.M.G.R. Medical University, Chennai. This has not been submitted previously by me for the award of any degree or diploma from any other university**.**

Place: Chennai DR.B.PARTHIBAN

Date :

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Institute of Child Health and
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dated:

The Institutional Review Board [Ethical committee] of Institute of Child Health and Hospital for Children, Chennai-08, was held on 30.01.2010 at 10.00AM at the Deputy Superintendents chamber.

Members Present: Dr.R.Kulandai Kasthuri
Chair Person.

Members:
1. Dr.K.Gita
2. Dr.P.Jeyachandran
3. Dr.D.Vijaya Sekaran
4. Prof.Girija Shyam Sundar
5. Mrs.Muthu Lakshmi, (Advocate)
6. Dr.P.Ramachandran
7. Mrs.Shubha Kumar

Member Secretary: Dr.Luke Ravi Chellaiah

Title: A Study of Evaluation of Faine's Criteria and IGM ELISA in the diagnosis of Leptospirosis".

The Institutional Review Board was satisfied with the revised format submitted by you. Hence the Institutional Review Board is pleased to approve the study.

Director and Superintendent.

To,
Dr.B.Parthiban,
Post Graduate,
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CONTENTS

INTRODUCTION

Leptospirosis is a worldwide public health problem. In humid tropical and subtropical areas, where most developing countries are found, it is a greater problem than in those with a temperate climate. It is difficult to confirm the diagnosis, because of lack of availability of cultures which is considered as the gold standard.[3] Wide variety of serological tests are available with varying sensitivity and specificity. ELISA IgM , IgM-specific dot-ELISA, LEPTO Dipstick, slide agglutination method, Dri-Dot assay, complement fixation assay, latex agglutination, indirect hemagglutination test, and indirect immunofluorescent test are to name a few.[1, 2, 4-12]Among these tests ELISA IgM has been considered the ideal one.[13]

Clinical manifestations are non-specific in the early stages of illness. The early institution of antibiotic therapy has been found to be beneficial in studies. [1, 2, 14] Faines criteria has been recommended by the world health organization as a useful clinical tool in the diagnosis of Leptospirosis.[15] However, there is paucity of data in literature regarding the usefulness of Faine's criteria in pediatrics. Hence, a study to assess the usefulness of Faines and modified Faines criteria has been attempted.

HISTORY

Adolf Weil first described Leptospirosis in 1886.[16] In 1888 Fieldler, named leptospirosis as “Weil's disease”. Huebner and Reiter and in 1915 demonstrated the organism in Germany and by Inado and Ido in Japan in the same year.[17] In 1918 Noguchi coined the name ‘Leptospira’ (thin spirals), followed by detailed microscopical examination and cultural studies. [18]

In 1917 the first isolate ‘icterohaemorrhagiae’ was named in Japan. Later in 1918 isolated serovar was ‘hebdomadis’ (non icteric) and in 1925 serovar ‘autumnalis’ from autumnal fever patient.

In the year 1925 in Indonesia serovar “bataviae” was isolated from an anicteric patient. Serovar “grippotyphosa” was the first animal isolate from cattle in USSR in 1928. In 1933 “canicola” from Netherlands, in 1937 “Pomona” and “australis” from Ballice , in 1944 “ballum” and “saxkoebing” and from Denmark and different other serogroups were subsequently isolated in various places of the world from different animal and reservoir hosts. Faine and Yanagawa and Faine showed that Leptospires were analogous to other bacteria in structure and that characteristic antigens were associated with structural elements in the year 1966. [19]

Morphology

The genus comprises of thin spiral organisms with 5-20 coils and hooked ends. This differentiates it from other spirochetes. A helically shaped cell cylinder and two periplasmic flagellae enable the organism to burrow into tissues. They arise from two sub terminally placed basal bodies. The helical configuration is right handed with more than 18 coils per cell. The organism stain poorly with aniline dyes and is best seen with fluorescent antibody and silver impregnation techniques (Fontana's stain and Levadity's stain) which stains them into a dark brown color against a yellow background.[20]

Leptospirosis is an infectious disease caused by leptospira interrogans complex, which has over 20 sero groups and more than 200 serovars. Rodents, domestic and wild animals form the reservoir of infection. Domestic animals such as cattle, dogs and pigs may act as carriers for several months (temporary carrier); rodents usually remain carriers throughout their life (permanent carrier). Thus rodents are considered the major reservoir of infection.[15]

Leptospires are excreted in the urine of infected animals and human beings are affected when they come in contact with the infected urine directly or indirectly, when exposed to infected soil and surface

water following monsoon rains. Therefore, the illness commonly occurs during the monsoon. The infection is probably transmitted when human beings walk through stagnant rainwater contaminated by urine of infected animals. Leptospires survive in dry soil for 6 hours where as in flooded conditions for 6 months. The organisms enter host through abraded skin of the feet during contact with infected water or through intact mucous membranes of eye, throat, and gut.

Leptospirosis can occur in both urban and rural areas. In urban areas due to overcrowding, improper sanitation and drainage facilities spread infection to both human beings and animals. Along with this presence of cattle, domestic rats, pigs, stray dogs, bandicoots, poorly maintained slaughter houses and habit of bare foot walking contribute to the spread of infection[21, 22]. Persons of all ages and races are susceptible.[3,23] The number of cases in a region often fluctuates from year to year due to various factors such as rainfall, flooding and animal infections. Boiling water, using Iodine tablets and UV sterilisers are very effective methods against killing leptospira.[29] One of the Methods stated by WHO for prevention of the disease is to interrupt the transmission route by providing clean drinking water. [30] In a study by Reis et al it was found that in people living in slum areas with less than 20 meters from an open sewage or garbage pit, the risk of contamination with *Leptospira*

was 1.4 times higher than others and poverty was found to be an independent risk factor for infection. For every household daily per capita income increase of one US dollar, there was a respective 11% reduction in the infection risk. [31]

World scenario

The number of human cases worldwide is not clearly known. With currently available reports, incidence ranges from approximately 0.1–1 / 100 000 / year in temperate climates and 10–100 / 100000 in the humid tropics. During epidemics and in risk groups, incidence may reach over 100 / 100000.[32]

Indian scenario

Unreliability of data is still a major problem in evaluating the presence and the actual incidence of Leptospirosis in many Asian countries including India.[33] A low index of suspicion of this disease coupled with the diversity and non-specificity of its presentation accounts for the significant number of cases that go un-recognized.[2] Published data available for Andaman Islands; one of the endemic areas of the world has a documented incidence rate of 50/100,000. [33]A sero-survey on humans by the Indian Council of Medical Research, Leptospirosis Task Force, indicated “high prevalence of leptospirosis” and endemicity in India.[34]

Although national incidence data is not available for India, Leptospirosis has been recognized as a major health problem. Natural disasters and poor sanitary conditions have contributed to the multiple epidemics and several outbreaks of the disease in the recent years.[35-39]

Mortality

The mortality rate in severe leptospirosis is in the range of 5-40%.. The mild form (90%) of the illness is rarely fatal. Immuno compromised and elderly people are at the highest risk. [40]

Pathogenesis

The pathogenesis of Leptospirosis is based of the following mechanism

1. Direct bacterial invasion
2. Non–specific inflammatory factors
3. Immunological reactions

Once they have gained entry, leptospires spread through the blood stream to all organs. Multiplication occurs in both blood and tissues. Within 24 hours, the organism can be isolated in most tissues except brain, skeletal muscles and aqueous humour. After 48 hours, they can be isolated from all tissues. Multisystem involvement results from bacterial

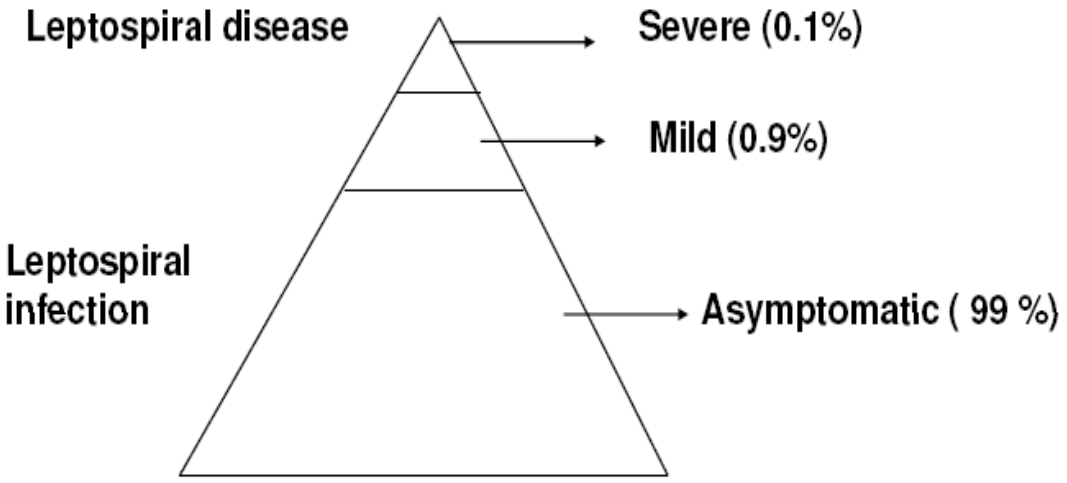
invasion and toxic reaction. Clearance of the organisms is accomplished by phagocytosis and humoral mechanisms. Leptospires rapidly disappear from the blood after the appearance of agglutinins. After the spirochetemic phase, which lasts from 4-7 days, the organisms can be recovered only from renal and ocular tissues. Leptospiruria continues for 1 to 4 weeks. [1, 2, 4]

Clinical manifestations

Leptospirosis can manifest in as many presentations as follows.[15, 21]

1. Anicteric Leptospirosis (~ >90%)
2. Icteric Leptospirosis (Weil's disease ~ < 10 %)
3. Hemorrhagic fever with renal syndrome
4. Atypical pneumonia syndrome
5. Myocarditis
6. Aseptic meningoencephalitis
7. Ocular manifestations
8. Unusual Clinical manifestations

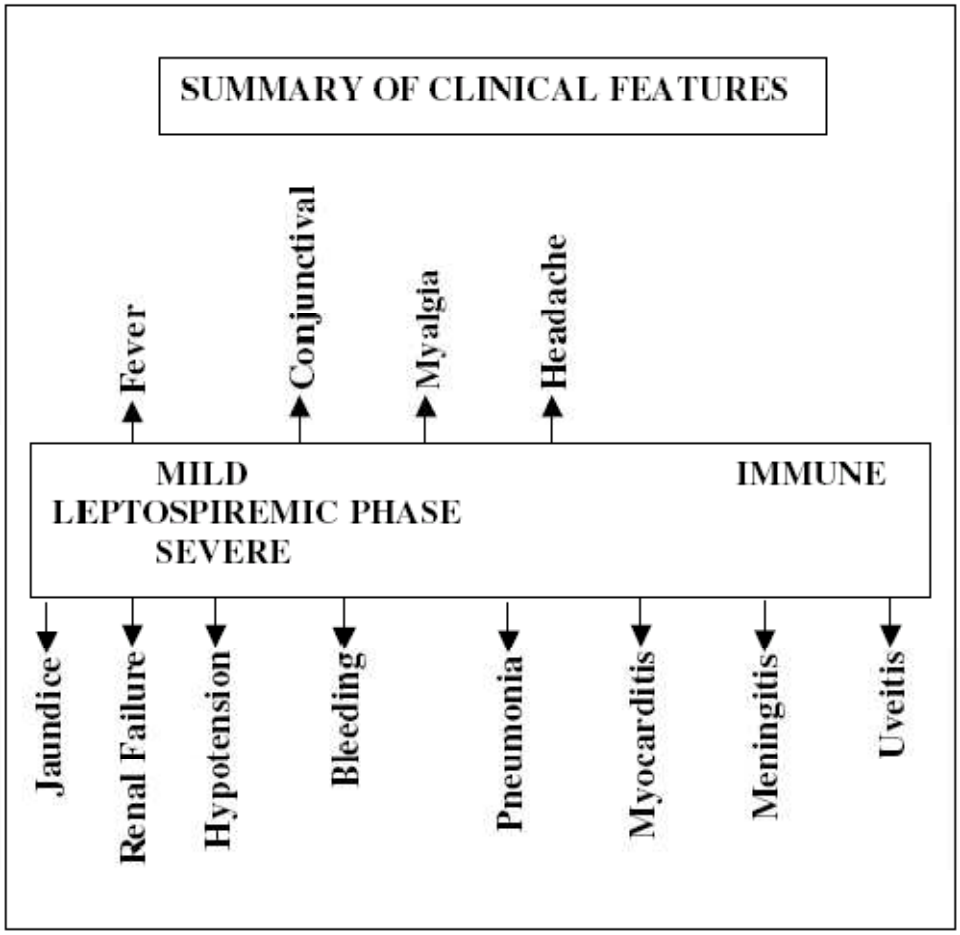
Clinical spectrum of leptospirosis



Incidence: 10 – 100 /100,000 population/year

Prevalence: 20-50 %

The incubation period is 7 – 14 days, but ranges from 2 – 21 days. In general, both anicteric and icteric cases follow a biphasic course, 'septicemia' or 'leptospiremic' phase.



Possible determinants of leptospirosis severity [41]

Virulence properties of infecting Leptospira
Infectious inoculum at the time of exposure
Preexisting immunity due to previous exposure
Hormonal influences (men more commonly develop severe disease)
Human host genetics: acquired or innate immunity
Nutritional factors: malnutrition, alcohol consumption

I. ANICTERIC LEPTOSPIROSIS

This can be mild with fever, headache, and body pains or more severe with a biphasic illness. The septicemic phase has an abrupt onset with chills, rigor, fever (temperature 39°C - 40°C), severe headache, and body pain. The pain and weakness make walking difficult. Severe pain in the back, neck, abdomen, and upper limbs are frequent. The headache is throbbing and often severe. Anorexia, nausea, and vomiting are frequent and may be associated with constipation or diarrhoea. Epistaxis may occur during the early stage. Chest pain, dry cough, and hemoptysis may occur. Mental symptoms such as restlessness, confusion, delirium, hallucination, and occasional psychotic behavior may occur.

The most characteristic findings on examination are conjunctival suffusion and severe myalgia. The conjunctival suffusion is described as reddening of the eye surface due to dilatation of the conjunctival vasculature with or without subconjunctival haemorrhage. It involves the bulbar conjunctiva only. A transient rash can occur. The ‘septicemia’ phase subsides in 4 – 7 days with temperature settling down. The second or immune phase is characterized by severe headache due to meningeal involvement, uveitis and low-grade fever. This lasts from 4 - 30 days or longer. The biphasic course may not be seen in all patients.

II. ICTERIC LEPTOSPIROSIS

In some patients, the septicemia phase instead of subsiding, progresses to a severe icteric illness with renal failure. Meningeal symptoms are frequent, but are overshadowed by hepatic or renal features. Severe bleeding, hypotension, cardiac and pulmonary complications are frequent. Death occurs usually due to renal failure. Sudden death may occur due to massive bleeding, arrhythmias, cardiac failure or respiratory failure. In those who are not severely ill, diuresis occurs and renal failure improves. The fever subsides and the general condition gradually improves in 2-7 days. Immunosuppressed patients may develop a fulminant course of leptospirosis. Two cases of Weil syndrome in transplant patients have been described. [42]

LIVER: Jaundice is the most important clinical feature indicating the severity of illness. Jaundice occurs between two to nine days and mostly between the fourth to sixth days but may, deepens rapidly, reaching a peak within a week. The liver is often enlarged and tender. Jaundice is mainly due to hepatocellular damage followed by intra hepatic cholestasis and rise in bilirubin load from absorption of tissue haemorrhage. Marked elevation of serum bilirubin with mildly elevated transaminases is characteristic.[43] Death is rarely due to hepatic failure.

KIDNEYS: Renal involvement is the commonest cause of death. Renal manifestations ranges from pyuria, granular casts, haematuria to severe renal failure. Renal manifestations are observed in all forms of disease irrespective of the disease severity or involved sero-group. In anicteric patients, microscopic haematuria, azotemia and mild proteinuria are noted.

Pre renal Azotemia, if uncorrected results in tubular necrosis. Acute interstitial nephritis also causes renal failure. Renal failure usually occurs in 14 days but may occur even as early as 4 days. Renal failure is transient and last for few days to 2 weeks on treatment.

HYPOTENSION: Hypotension is an important complication, noted in patients with severe Leptospirosis. The causes of hypotension are 1) Hypovolemia secondary to poor intake of feed, vomiting, and insensible water loss due to fever 2) massive haemorrhage mostly gastrointestinal 3) unidentified vasoactive endotoxin 4) widespread vascular injury leading to fluid shifts from intravascular to extravascular fluid spaces 5) myocardial dysfunction 6) adrenal hemorrhage (rarely).

III. ATYPICAL PNEUMONIA SYNDROME

Severe hemorrhagic pneumonitis may occur usually in the second week, but occasionally as early as 24 – 48 hours after onset. This may present with hemoptysis, chest pain, respiratory distress and cyanosis.[26] Massive hemoptysis may cause asphyxiation. Radiological abnormalities range from single ill-defined opacity, multiple areas of infiltration to a large area of consolidation. This resolves within 2 weeks without any residual damage.

IV. HAEMORRHAGIC FEVER WITH RENAL SYNDROME

Bleeding is a constant feature of Leptospirosis and is due to vascular damage.[2] It is usually mild in anicteric cases but more common in a severely icteric patient. Bleeding may occur from respiratory, alimentary, renal or genital tracts and occasionally into subarachnoid space and adrenal glands. Death may occur from massive bleeding usually gastrointestinal or into internal organs. This may be associated with renal failure.

V. MYOCARDITIS

Cardiac complications are frequent in severe Leptospirosis. They are usually mild and are observed as electrocardiographic abnormalities

ranging from low voltage complexes, non-specific ST and T wave changes, conduction defects and arrhythmias. Atrial fibrillation is the most common arrhythmia observed.[44] Severe manifestations such as cardiomegaly, cardiac failure and severe arrhythmias due to hemorrhagic myocarditis are observed. Sudden death may occur from cardiac failure or arrhythmias. All cardiac abnormalities revert to normal within 2 to 3 weeks. Other reported cardiac abnormalities include myocarditis and AV block in 44% of patients with leptospirosis.[45, 46]

VI. OCULAR MANIFESTATIONS

Conjunctival suffusion is a common feature of the septicemic phase and is usually associated with conjunctival hemorrhage. There is no inflammatory exudate and true conjunctivitis does not occur. It usually occurs in the first three days and lasts for one day to more than a week. It subsides within a week without any complications. More important is the late complication of anterior uveal tract inflammation which presents clinically as iritis, iridocyclitis and rarely as chorioretinitis. This may occur as early as the second week or may be delayed up to a year but is more frequent in the first 6 months. Uveitis may be unilateral or bilateral and the course is variable (i.e. acute benign episode, recurrent episodes or a chronic process). The ultimate prognosis is good but chronic Uveitis may cause blindness by cataract formation and hypopyon in the anterior

chamber. An immunological basis for uveitis is suggested by the prolonged persistence of leptospires in the ocular fluid and the demonstration of agglutinins in the aqueous humour. [47]

VII. ASEPTIC MENINGOENCEPHALITIS

This usually occurs in the immune phase and may present with signs of meningeal irritation. The CSF shows lymphocytic pleocytosis, raised proteins (1–2gm/L) and normal sugar. Convulsions, focal neurological deficits, myelitis, polyneuritis and encephalitis are rare. The fact that leptospires are isolated consistently from the cerebrospinal fluid but disappear during the onset of meningeal signs following antibody formation suggests that, immunological mechanism is responsible for the development of meningitis. Prognosis in meninigitic illness is excellent.

VIII. UNUSUAL CLINICAL MANIFESTATIONS

Musculoskeletal symptoms

A fatal case of rhabdomyolysis was reported by O'Leary et al.[51] Skeletal muscle involvement independently correlates with the severity of disease.[52]

Gastrointestinal manifestations

Pai and Adhikari reported a rare case of pancreatitis following Leptospirosis.[48] Monno and Mizushima reported a rare case of acute

acalculus cholecystitis in a patient with infection due to serovar Autumnalis.[49] Peritonitis is another rare manifestation of leptospirosis.[50]

Hematological manifestations

Somers et al reported a rare case of erythroid hypoplasia in a case of Leptospirosis.[53] Rare immune-mediated manifestations of leptospirosis include antiphospholipid antibody syndrome and reactive arthritis.[54, 55]

Endocrine abnormalities

Panidis et al described a rare case of male hypogonadism, following leptospirosis presumably related to hormone deficiency at the hypothalamo-pituitary level. [56] Abnormalities in hormonal secretion have been found in experimental infection in animals but there is paucity of data regarding the incidence and effects of such abnormalities following infection in humans. [57]

DIFFERENTIAL DIAGNOSIS

Leptospirosis with its varied manifestations mimics a large number of disease processes. In patients with fever , generalized myalgia and head ache,[15] in first few days of fever conditions like enteric fever,

malaria, viral hepatitis, viral fever, viral hemorrhagic fever, sepsis, meningitis and encephalitis should be the differential diagnosis. [3, 4, 58, 59]

Anicteric leptospirosis is usually misdiagnosed as PUO, viral fever, malaria, enteric fever, influenza or pyelonephritis.

Severe Icteric leptospirosis may be confused with febrile icteric illness like viral hepatitis, septicemia with jaundice and malaria. Severe headache, myalgia and conjunctival suffusion are constant features, and proteinuria is common and the onset is abrupt in leptospirosis, whereas in viral hepatitis, onset is gradual, headache and myalgia are mild and proteinuria and conjunctival suffusion are absent. Jaundice also occurs in malaria and sepsis.

Leptospiral renal failure should be differentiated from renal failure due to malaria, sepsis and hanta virus.

In those presenting with meningitis, leptospirosis has to be differentiated from bacterial and viral meningoencephalitis. Bacterial meningitis can be confirmed by spinal fluid examinations. Viral meningitis is indistinguishable from Leptospiral meningitis.

Conjunctival suffusion, myalgia and evidence of bleeding suggest the diagnosis of Leptospirosis and this can be confirmed by the serological tests.

DIAGNOSIS OF LEPTOSPIROSIS

The diagnosis is established most often by serologic testing and less frequently by isolation of the infecting organism from clinical specimens. Serologic tests for leptospira include genus specific and serogroup specific tests. The reference method is the microscopic agglutination test. [1] The diagnosis is difficult to confirm, it may be confused with other diseases, the disease may be mild and not be investigated in the laboratory or laboratory tests may not be available or the available tests have low sensitivity during early phase of disease as these tests detect antibodies.[60, 61]

Recommended case definition (WHO) [32]

Laboratory criteria

Presumptive diagnosis

A positive result of a rapid screening test such as IgM ELISA, latex agglutination test, lateral flow, dipstick etc.

Confirmatory diagnosis

1. Isolation of pathogenic Leptospires from blood or other clinical materials through cultures.
2. A positive PCR result using a validated method (primarily for blood and serum in the early stages of infection.
3. Fourfold or greater rise in titer or seroconversion in microscopic agglutination test (MAT) on paired samples obtained at least 2 weeks apart. A battery of Leptospira reference strains representative of local strains should be used as antigens in MAT.

Case classification

Suspected:

A case that is compatible with the clinical description and a presumptive laboratory diagnosis.

Confirmed :

A suspected case with a confirmatory laboratory diagnosis.

CDC CRITERIA [62]

Laboratory criteria for diagnosis

- Isolation of Leptospira from a clinical specimen or
- Fourfold or greater increase in Leptospira agglutination titer between acute and convalescent phase serum specimens obtained greater than or equal to 2 weeks apart and studied at the same laboratory or
- Demonstration of Leptospira in a clinical specimen by immunofluorescence

Case classification

Probable:

A clinically compatible case with supportive serologic findings (i.e., a Leptospira agglutination titer of greater than or equal to 200 in one or more serum specimens)

Confirmed:

A clinically compatible case that is confirmed by laboratory

SEROLOGY:

The serological tests for diagnosis of Leptospirosis are classified as genus specific tests and serovar specific tests.

Genus specific tests: The common tests are the ELISA and Macroscopic slide agglutination tests (MSAT). The other tests are latex agglutination test, complement fixation test and haemagglutination tests. The genus specific tests are the investigation of choice for current infection. These tests are simple, more sensitive and become positive earlier than MAT.

ELISA: ELISA detects genus specific IgM antibodies, which will be positive in fourth or fifth day of illness. [4, 6] Detection of IgM antibodies are usefull in rapid diagnosis of current infection. The test is extremely sensitive that all the materials used are carefully cleaned. It is best if the glassware is reserved exclusively for this purpose only. The ELISA test becomes positive a little earlier than the MAT because it is more sensitive to IgM antibodies. However, the test is not infallible and may be negative, e.g. in a large percentage of infections caused by serogroup Grippotyphosa and to a lesser extent, in the detection of serogroup Australis infections. If a variety of strains from different serogroups are used as antigens instead of an antigen derived from the Patoc I saprophytic strain, the sensitivity of the test is increased.[32]

MSAT: The slide agglutination test is a simple macroscopic test in which a drop of the dense suspension of leptospira is mixed with a drop of serum on a slide and examined by dark field microscopy. These tests have good sensitivity up to 85%. [2, 4-12]

SEROVAR SPECIFIC TESTS

Microscopic agglutination test (MAT)

MAT is the gold standard test for diagnosis of Leptospirosis which is unsurpassed for its diagnostic specificity. [1, 7, 9] The main advantage is that serovar can be identified which is of epidemiological importance. The test is complicated as it involves the use of a battery of leptospira of widely differing antigenic structure to cover the spectrum of leptospiral infection. Therefore, it requires the maintenance of stock cultures. Rising titers or an initial high titer is diagnostic of Leptospiral infection. These titers begin to rise by the end of the 2nd week and peaks in 3rd to 4th week, therefore are not valuable for the diagnosis of current infection. [1, 2]

Since the agglutinins stay for prolonged period after infection, a proportion of the healthy individuals will have detectable levels of antibodies. At the same time, in true patients it takes some time for the antibodies to reach detectable levels. These two sets of persons account for the false positive and false negative results of the test respectively.

The former depends upon the endemicity of the disease in an area and hence the endemicity is a factor that has to be considered into account while fixing a cut off titer for optimal accuracy of the test. Reference laboratories often face this problem as they receive samples from areas of different endemicities.

The high titers persists for long time, which helps in epidemiological surveys, but high titers from previous infection interferes with the diagnosis of recent infection. Considerable effort is required to reduce the subjective effect of observer variation, even within laboratories. The importance of determination of base line titers in the community hence cannot be overemphasized.[69]

The difficulties in utilizing MAT are due to the following factors.

a. The antibody titers raise and peak only in 2nd or 3rd week, making it a less sensitive test. A study of 108 cases of leptospirosis from Brazil have revealed that 65% of the first sample were positive by MSAT compared to 44% by MAT.[45]

b. A four-fold rise in titer or seroconversion is the most definitive criteria for diagnosis of leptospirosis. [1, 3, 32, 62] Therefore a second sample is mandatory, which is difficult to obtain. In such circumstances, a single high titer in MAT can be taken as diagnostic criteria. As MAT titers peak

and persist for a long time (5 - 10 years), they would interfere with current diagnosis. A titer of 1:80 is considered significant, but there is controversy on the single diagnostic titer as they depend on endemicity. In endemic areas, a titer of 1:80 or 1:160 is considered low; while high titer is usually > 1:320 (some consider 1:640 or 1:1280 as diagnostic criteria). In non- endemic areas, 1/80 titer is taken as the diagnostic criteria. It is preferable to do rapid tests along with single high titers. Positive rapid tests with high titers suggest current infection while a negative rapid test is probably due to past infection. In Andaman, a titer of 1:160 is considered as diagnostic titer because of high endemicity.

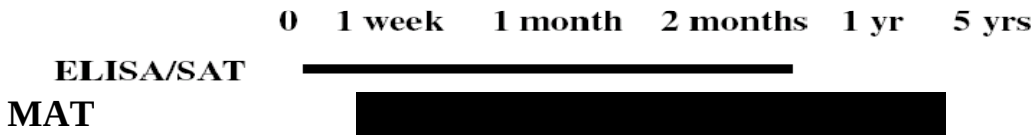
c. The test is complicated requiring dark field microscopy and cultures of various live serovars. This may not be available in small laboratories.

d. Continuous risk of cross-contamination of the antigen cultures, necessitating periodic verification of each serovar.

e. Repeated weekly subculture of large numbers of strains presents hazards for laboratory workers

Interpretation of Tests

ELISA/SAT	MAT	INTERPRETATION
+	+	Current Infection
+	-	Current Infection
-	+	Past infection
NA	Rising titres	Current Infection



Other Tests

POLYMERASE CHAIN REACTION

PCR is promising in both sensitivity and specificity, but is complicated and expensive. Its value for rapid diagnosis is being evaluated and is used in higher centers to detect leptospires in body fluids like serum, urine, aqueous humour or culture supernatants. [2, 4, 5, 63-66] PCR can rapidly confirm the diagnosis in the early phase of the disease, when bacteria may be present and before antibody titers are at detectable levels. Positivity of IgM is 61% in cases of leptospirosis diagnosed by PCR. [67] PCR analysis of urine can be more successful for early diagnosis of Leptospirosis than PCR analysis of serum. [68]

PCR requires special equipment, dedicated laboratory space and high skilled personnel. Conventional PCR may give false-positive results in the presence of minute amounts of extraneous DNA that may

contaminate working areas. It may also give false-negative results because inhibitors are present in the clinical materials that are being examined. Although PCR technology is now widely used for the diagnosis of many diseases, its general value for the rapid diagnosis of Leptospirosis has not been evaluated worldwide as it is not yet widely used, particularly in tropical and subtropical countries. PCR is most sensitive in initial disease phase but is less sensitive than the serological tests over the course of the disease.

Culture:

The isolation of Leptospirosis by culture of blood, CSF and urine is the most definite way of confirming the diagnosis of Leptospirosis. Unfortunately, culture of blood does not contribute to an early diagnosis as results appear late, weeks or even months after inoculation of culture medium. Leptospires are obligatory aerobes , cultivated in a suitable medium at 30ºC, and are susceptible to acidic medium so need an optimum ph 7.2-7.4.Their generation time varies between 7–12 hours.

The nutritional requirements of Leptospira are unique. They can be grown in artificial culture media. They require Vitamin B_1, B_{12}, long chain fatty acids and utilize purines, but not pyrimidines. Fatty acids are provided by incorporating 10% rabbit serum or 5% bovine albumin into

the medium. [1, 2] The Culture medium is hazardous to the health workers in research lab. Culture is rarely carried out in routine laboratories and this facility is available only in research centre.

Dark-field Microscopy (DFM)

Demonstration of leptospires by dark ground microscopy (DGM) during Septicemic phase (Leptospiremic phase) is a simple technique. Dark ground microscopy is less expensive and simple. Microscopic examination of the centrifuged urine using dark-field illumination is a convenient and rapid diagnostic test. With a sterile bottle midstream urine sample is collected and stored at 4°C and transported to the laboratory as soon as possible. [70]

Dark-field microscopic (DFM) examination requires a skilled observer to differentiate the typical movements of Leptospira from artefacts. It should be considered that Leptospires are very fragile and sensitive to the pH of the urine, so with one half of sample add 1 drop of formalin to 20-30 ml of urine and the other half submitted in the fresh state. Formalin prevents bacterial overgrowth and the fresh urine sample can be used for culture. The sample is negative if no spirochete observed in 100 fields in each of the preparations.

MANAGEMENT

Chemotherapy:

The aim of chemotherapy is to eradicate Leptospirosis and to prevent complications. Leptospirosis is sensitive to most antibiotics. Antibiotic administration before the 7th day of fever reduces hospital stay and severity of illness. [1, 76] In paediatric age, antibiotic administration even after 7 days tends to reduce the complications like renal failure and thrombocytopenia . [76] Penicillin or tetracycline (in children > 9 years of age) should be started as soon as the leptospirosis is suspected. [1,2,4,5,23,58,59, 64, 77-79] Penicillin is the most effective antibiotic when given early. Parenteral penicillin G 6-8 million U/m^2/day given through intravenous route in 6 divided doses for 7 days is the drug of choice. [1,2] Tetracycline 10-20 mg/kg/day orally or intravenously in 4 divided doses for 7 days (in children more than 9 years) can be used in those allergic to penicillin.[1,2,5,77] Fever subsides in 24 – 36 hours. Ampicillin 25-50mg/kg/dose Q 6th hourly or oral amoxicillin (25-50 mg/kg/day three times a day for 7 days) is an alternative therapy for children < 9 years of age. [1, 2,5,77] Erythromycin 30 mg/Kg/day divided tid is effective. Ciprofloxacin has been occasionally used, especially in patients with uveitis , the use of ciprofloxacin need more clinical trials. [64, 79] Ceftriaxone and penicillin G were equally effective for the treatment of

severe leptospirosis. Once-daily administration and the extended spectrum of Ceftriaxone against bacteria provide additional benefits over intravenous penicillin in institutes where typhoid and Leptospirosis may co exist or the disease resemble the above. [80]

Symptomatic and supportive treatment: Of primary importance is the meticulous attention to hydration status and maintain normal hemodynamic status by using intravenous fluids to avoid pre renal azotemia. Patients unresponsive to therapy should be managed as established renal failure. Headache and myalgia are treated with analgesics, fever with anti pyretics, restlessness and anxiety with sedatives and anemia with blood transfusion. Treatment of cardiovascular collapse, and provision of dialysis for renal failure, is equally important. [2, 4, 58, 73, 81]

Peritoneal dialysis has been found to be safe, simple and effective procedure for management of Leptospiral renal failure. If peritoneal dialysis is contraindicated , hemodialysis can be done.

PROGNOSIS

Anicteric leptospirosis carry good prognosis, rarely mortality due to fatal pulmonary hemorrhage and myocarditis occurs.weil's disease have mortality rate was upto 15%.[1,2,4,58,82] overall mortality in

leptospirosis is about 15-40% which can be reduced to 5% by proper management with appropriate antibiotics. Major cause of death is renal failure followed by massive bleeding and cardiac complications.

It should be realized that clinical data on milder (Anicteric) forms of Leptospirosis is inadequate in our country and this can be made available only if simpler tests are done in small laboratories. There is paucity of data in either Faine’s or modified Faine’s criteria in children so far.

REVIEW OF LITERATURE

Faine's and modified Faine's criteria:

A-Faine Solomon in 1982, at Geneva in the WHO offset publication; no. 67, formulated a criteria for diagnosis of Leptospirosis on the basis of clinical, epidemiological and laboratory data. It was followed worldwide to diagnose Leptospirosis. The standard Faine's criteria had a sensitivity of 41.9%, specificity of 84.9% and a positive predictive value of 41.9% in study by Shivakumar et al.[72] Faine's criteria had a sensitivity of 88.9%, specificity of 80.2 %, positive predictive value of 30.8 % and negative predictive value of 98.6% by Rao et al.

B-In a study by Shivakumar et al.[72] 106 patients with positive Faine's criteria Were analyzed. There were 69 males & 37 females. Mean age was 31.2 years. Outdoor manual workers (39.4%) were at risk of developing leptospirosis. Contaminated environment (95.2%), animal contact (94%) & rainfall were the important epidemiological risk factors. Fever, headache & myalgia were the common clinical features. Jaundice (17.8%) & renal failure (10.3%) were the important complications. Anicteric leptospirosis (82.2%) was the common presentation. Mortality was nil.

Part A has clinical signs and symptoms, part B epidemiological factors and part C has laboratory criteria for endemic and non endemic areas.

The scoring is done and diagnosis is made based on the following

Presumptive diagnosis of Leptospirosis is made if

Part A or part A & part B score is 26 or more

Part A, B, C (Total): 25 or more

A score of 20 to 25 suggests Leptospirosis as possible but unconfirmed diagnosis.

Faine's Criteria		Modified Faine's Criteria	
Part A : Clinical Data		**Part A : Clinical Data**	
Question	**Score**	**Question**	**Score**
Headache	2	Headache	2
Fever	2	Fever	2
Temp>39^0C	2	Temp > 39^0C	2
Conjunctival suffusion	4	Conjunctival suffusion	4
Meningism	4	Meningism	4
Muscle pain	4	Muscle pain	4
Conjunctival suffusion Meningism Muscle Pain	10	Conjunctival suffusion Meningism Muscle Pain	10
Jaundice	1	Jaundice	1
Albuminuria / Nitrogen Retention	2	Albuminuria / Nitrogen Retention	2
Total Score		Total Score	
Part B : Epidemiological factors Contact with animals or Contact with known Contaminated water	10	Part B : Epidemiological factors Rainfall Contact with contaminated Environment Animal contact Total	 5 4 1 10
Part C : Bacteriological and Lab Findings Isolation of leptospira in culture – Diagnosis certain Positive Serology (MAT) Leptospirosis Endemic		Part C : Bacteriological and Lab Findings Isolation of leptospira in culture – Diagnosis certain Positive Serology	
Single positive – Low titre Single positive – High titre Leptospirosis Non Endemic Single positive – Low titre Single positive – High titre Rising titre (Paired Sera) **Total**	2 10 5 15 25	ELISA IgM Positive MSAT – Positive MAT – Single High titre Rising titre (Paired Sera) **Total**	15 15 15 25

MODIFIED FAINE'S CRITERIA

Certain modifications have been made in the epidemiological (Part B) and the laboratory criteria (Part C) of original Faine's criteria by Shivakumar et al to make the diagnosis more practical in Indian institutions. [74] In the Modified Faine's Criteria rapid tests (ELISA / MSAT) have been introduced in Part C and Rainfall has been included in Part B to make the diagnosis early and simple.[74] Modified Faine's criteria had a sensitivity of 58%, Specificity of 97.4% and positive predictive value (PPV) of 85.7% in study by Shivakumar et al. [74] This criteria is being utilized for diagnosis of Leptospirosis in district and teaching institutes.[75]

During an epidemic, the microbiology laboratories would be burdened with large number of samples for MAT. It would be impossible to do MAT for large number of samples as it is a complicated test. In addition the laboratories need to have all the serogroups, otherwise, a negative MAT does not exclude current Leptospirosis, if the considered serogroup is not available. Therefore, ELISA/MSAT is adequate for current diagnosis.

History of animal contact (Part B) is not essential for diagnosis of Leptospirosis in developing countries. The more important epidemiological factors in our country are 1. Rain fall 2. Contact with contaminated environment. During rainfall, those who come into contact

with water contaminated with infected rodents (or other animals) urine are prone to develop Leptospirosis which is facilitated by environmental factors. It is impossible to trace the source of infection and any person can be infected, irrespective of direct contact with animals.

Thus, in the early stages of infection (5 days), clinical features are very important to suspect Leptospirosis utilizing Faine's criteria (Part A). But the diagnosis should always be confirmed by ELISA (or) MSAT. It is recommended that Leptospirosis diagnosis can be done by making the following modification of Faine's criteria. [81]

FAINE'S CRITERIA

PART A — No modification

PART B — SCORE

The score of 10 in Part B has been split into

1.	Rainfall	5
2.	Outdoor contact with contaminated environment	4
3.	Animal contact	1

PART C (> 5 days)

a) Positive ELISA/MSAT

The reasons for the modifications are

1. Laboratory tests are essential for diagnosis. ELISA IgM/MSAT are adequate for the diagnosis of current infection. If MAT were available, rising titers would confirm the diagnosis and identify the serovars

2. Epidemiological factors such as rainfall and contact with contaminated environment are important for diagnosis. Most of the cases of Leptospirosis are reported in the monsoon or post monsoon season.

3. Clinical features if combined with epidemiological and laboratory data confirm the diagnosis of Leptospirosis.

C- In a study by Sunil Sethiet al and Navneet Sharma et al in PGIMER showed increased incidence from 11.7% in 2004 to 20.5% in 2008 as diagnosed by IgM elisa and MAT in paired sera. The incidence showed peak during rainy season. In this study modified faine's criteria diagnosed 76 cases (88.3%)

D- In a study by V chayhan et al ,in adult population . Predominant complaints were fever, headache, jaundice and myalgia. with history of contact with contaminated environment or animals, with abnormal renal and liver parameters. Ten were positive for IgM Elisa with 2 borderline positive Elisa . One PCR was positive method. Ten had Weil's syndrome was the presentation for ten patients, one had acute respiratory distress syndrome (ARDS) who went for ventilator care. No deaths reported in this study. All were treated with intravenous ceftriaxone and oral

doxycycline. Applying Faine's criteria was positive in 7 and modified Faine's criteria was positive in 13 .

E-In a study by Smitha et al 2004-05 Jipmer department of microbiology Jipmer Pondicherry with a Sample size of 110. Seropositive rate of leptospirosis in and around Pondicherry is 36.3% by serovar specific MAT test and the prevalent serovar found is icterohaemorrhagicae,Pomona and pyrogen.This study shows both IgM elisa and MAT test were useful for the diagnosis of leptospirosis in suspected cases. Their study also shows the seroprevalance rate of leptospirosis in pyrexia of unknown origin cases in and around Pondicherry.

OBJECTIVES

To assess the sensitivity and specificity of Faine's, Modified Faine's criteria and IgM Elisa in diagnosing pediatric Leptospirosis.

STUDY JUSTIFICATION

Leptospirosis is a common zoonoses which is under reported and under diagnosed in India. It has been reported from kerala, Maharashtra, Andaman, Tamilnadu and Gujarat. It is not reported from other areas due to lack of diagnostic facilities. The problem of under diagnosis is because of complicated diagnostic tests. MAT is gold standard test. But it is complicated, less sensitive & requires 2 samples for diagnosis. MSAT & ELISA has become available which has made diagnosis easy.

Clinical manifestations are non-specific in the early stages of illness. The early institution of antibiotic therapy has been found to be beneficial in studies. [1, 2, 14] Faines criteria has been recommended by the world health organization as a useful clinical tool in the diagnosis of Leptospirosis.[15] However, there is paucity of data in literature regarding the usefulness of Faine's criteria in pediatrics. Hence, a study to assess the usefulness of Faines and modified Faines criteria has been attempted.

MATERIALS AND METHODS

Methodology

Study Design - Descriptive study/ Evaluation of study design.

Study place - Institute of Child Health and Hospital for Children, Egmore, Chennai (ICH)

Study period - January 2010-october 2011.

Study population - 91

Inclusion criteria

Children from 1year to 12 years of age hospitalized for fever of more than 5 days duration and signs suggestive of leptospirosis with any two of the following features. [15]

1. Headache
2. Myalgia / Muscle tenderness.
3. Conjunctival suffusion
4. Features of meningitis
5. Jaundice

Exclusion criteria

Fever with obvious foci like Abscess , Cellulitis , Lymphangitis or any child with a confirmed diagnosis other than Leptospirosis at the time of discharge.

Methodology

Children satisfying the inclusion criteria were subjected to detailed history and thorough physical examination by a single observer. Faine's and modified Faine's scoring was carried out at the time of admission. Proforma was filled up. Three ml of blood sample is obtained by clean venipuncture with asepsis in a sterile syringe and following investigations were carried out Leptospira IgM Elisa, MSAT and MAT (Apart from the routine investigations). Blood obtained by venipuncture was allowed to clot at room temperature (20-25°C) and then centrifuged according to the National Committee for Clinical Laboratory Standards (NCCLS)

MSAT, MAT and Leptospira IgM Elisa test was carried out in (Panbio- kit) were done in department of Microbiology, Madras Medical College, Chennai. The test was performed by the same technician, once a week by pooling the sample.

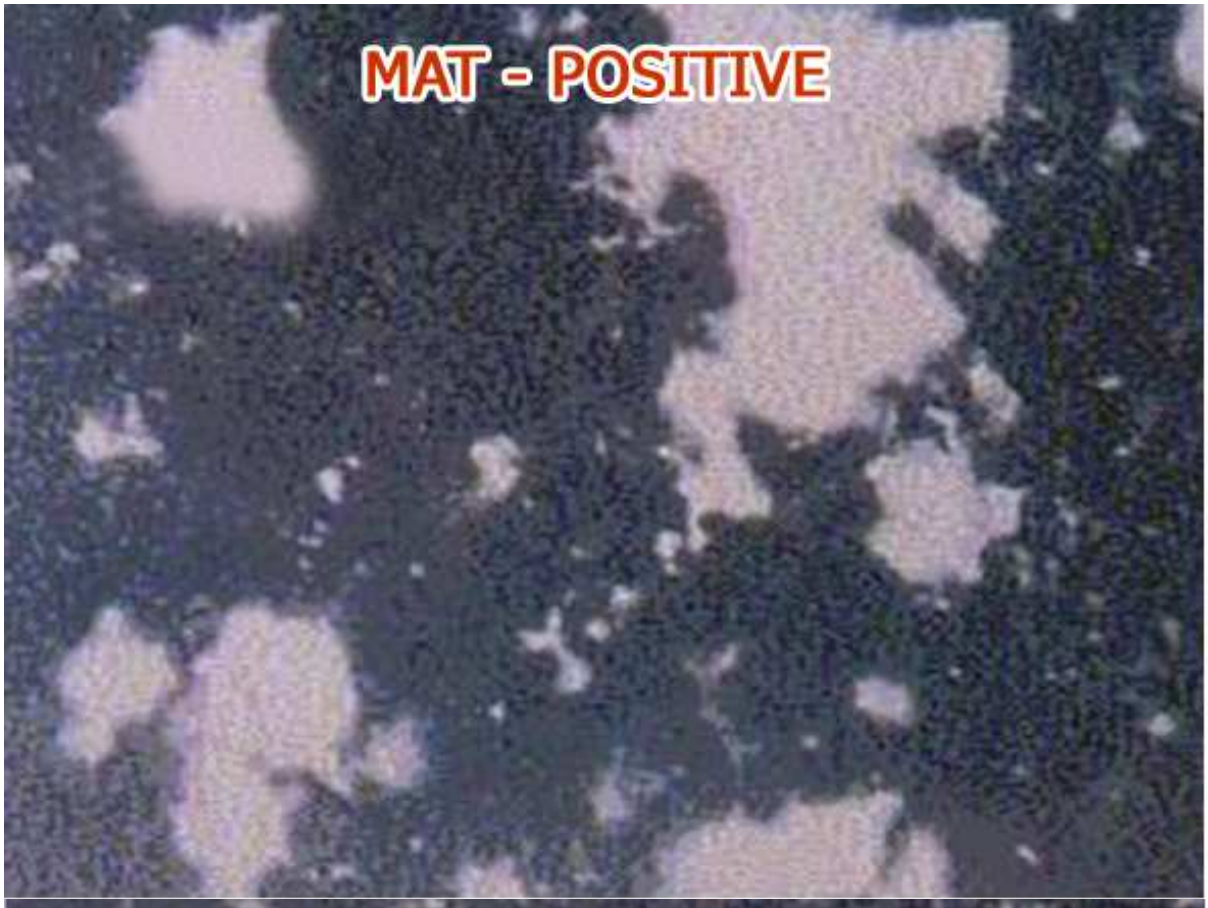
The Leptospirosis laboratory, Leptospirosis research cell was established in July 1994 at the Institute of Microbiology, Madras Medical College.

The other tests performed were, hemoglobin, total and differential counts, packed cell volume, peripheral smear study, platelet count, smear for malarial parasite, erythrocyte sedimentation rate, blood widal, liver function tests, creatinephosphokinase, electrolytes, urea, creatinine, blood

- enteric and non-enteric culture, CSF analysis, chest x-ray and ultrasound abdomen as necessary . Second sample of blood was taken after 2 weeks of the 1st sample for paired sera analysis of MAT either before discharge or at review, whichever is feasible.

MICROSCOPIC AGGLUTINATION TEST

The MAT was performed with nine live culture antigens (Icterohemorrhagiae, Australis, Autumnalis, Hebdomadis, Grippotyphosa, Canicola, Pomona, Patoc and Bataviae) using standard microtiter methodology. The sera were initially screened at dilutions of 1:20 and those that were positive were titrated further to the endpoint. The highest dilution of serum that agglutinated 50% of leptospires under dark field microscopy was presumed to represent the titer of antibody specific for the particular serogroup used. When two or more serogroups reacted at the same (highest) titer, the result was recorded as mixed equal. Controls were put up for each one of the battery of antigens used in the test. An initial titer of greater than or equal to 1: 160 or a four- fold rise in titer of MAT was considered significant for the diagnosis of Leptospiral infection.


MAT - POSITIVE


MAT NEGATIVE

Interpretation of results

The highest dilution of serum antigen mixture, showing 50 % agglutination, is taken as the end titer of the serum for that particular antigen.

MACROSCOPIC SLIDE AGGLUTINATION TEST

Procedure

One drop (5 µL) of antigenic suspension was mixed with equal amount of serum (both heated and 1:10 diluted), on a depression slide and rotated on a rotator at 180 rpm for four minutes. It was examined macroscopically for the presence of agglutination.[83] Positive and negative controls were also put up.

Interpretation of results

The results were reported as negative, 1+, 2+, 3+ and 4+ based on the percentage of agglutination. In our study we took 2+ and above as positive

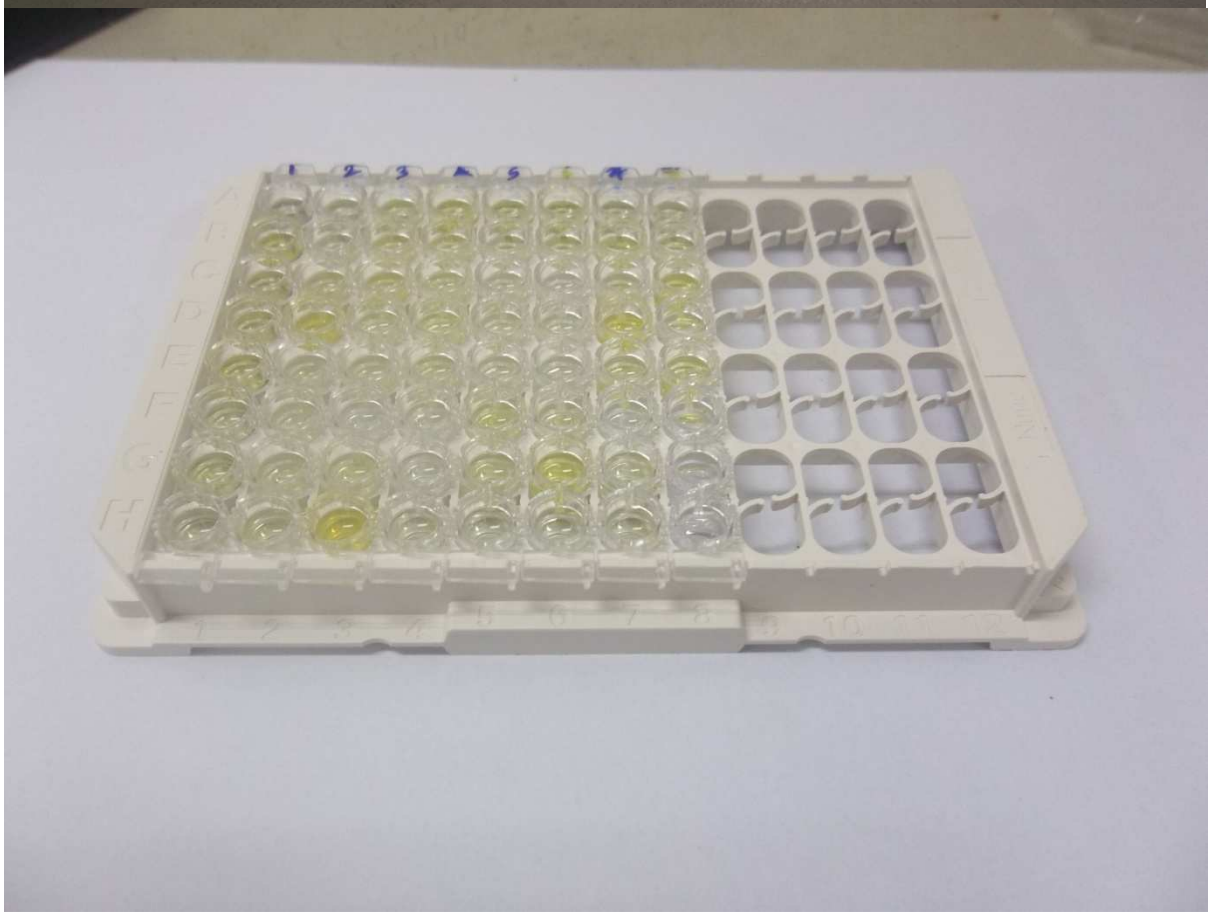
MAT and MSAT were done by a single technician and by standard procedures.[84]

Criteria for diagnosis

A titer more than 11 Panbio units by IgM Elisa was considered as diagnostic of leptospiral infection for the purpose of the study.[85]

IgM ELISA


panbio
LEPTOSPIRA
IgM ELISA
IVD In Vitro Diagnostic Use
REF
E-LEP01M
LOT
10291
2012-02
2-8°C



Protected water for the purpose of the study was defined as either boiled water, UV treated, reverse osmosis process done or packaged mineral water. The remaining type of water supply was taken as unprotected water supply in this study. Monsoon season is considered from July to December. Animal contact was defined as rearing a pet animal like dog, cat or cattle at home or sighting rats at their home. Rainfall is scored if there is any rainfall within 21 days of the illness in their locality. Jaundice was given a score if there is clinical jaundice or laboratory evidence of bilirubin > 2.5 mg/dl. Albuminuria is considered as 1+ or more proteinuria by urine dipstick. Nitrogen retention is scored 2 points when urea levels were > 40 mg/dl. Either albuminuria or nitrogen retention were scored. Chennai is considered as endemic area for the leptospirosis. [34]

ELISA – IgM

Panbio Leptospira IgM ELISA test (Brisbane, Australia) was used for qualitative and quantitative detection of human IgM antibodies directed against pathogenic Leptospira. Micro titer plates coated with antigen, constituted the solid phase. The kits were stored at 2-8°C in sealed aluminum bags with desiccant. The procedure was carried out as per the manufacturer's instructions.

The test was evaluated for each kit with the absorbance readings of the control sera and the calibrator sera and comparing with the acceptable

values of these sera found on the accompanying specification sheet weekly.

Results

Positive result	:	> 11	Panbio units.
Low positive result	:	9 - 11	Panbio units.
Negative results	:	< 9	Panbio units

Statistical Analysis

Sample size was calculated on the basis of expected proportion 21% with confidence interval 84% and alpha error 0.05 and calculated as 91 with variation 40%.

Proportion, mean and standard deviation (mean + or – sd) of the outcome variables as applicable will be arrived at . Faine's criteria and IgM ELISA in the diagnosis of leptospirosis will be assessed by comparing their results with the gold standard of MAT by arriving at sensitivity ,specificity ,positive predictive value ,negative predictive value and overall accuracy

RESULTS

A total of 91 children, who fulfilled the inclusion criteria were studied as per protocol. With a mean age 5.412 years 57 children were male and 34 were female gender.

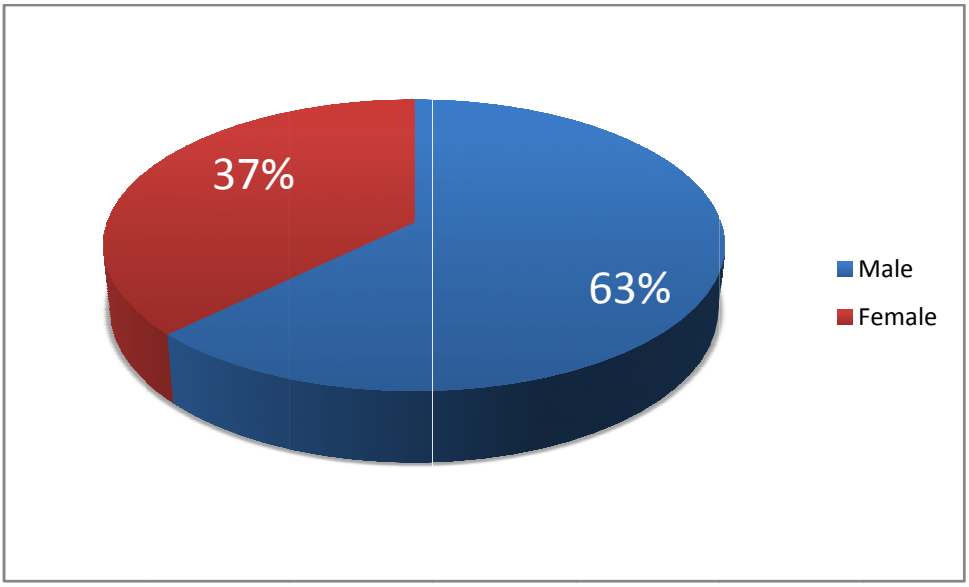


Figure 1: Gender distribution

The above figure shows that 63% were male gender and remaining 37% were female**.**

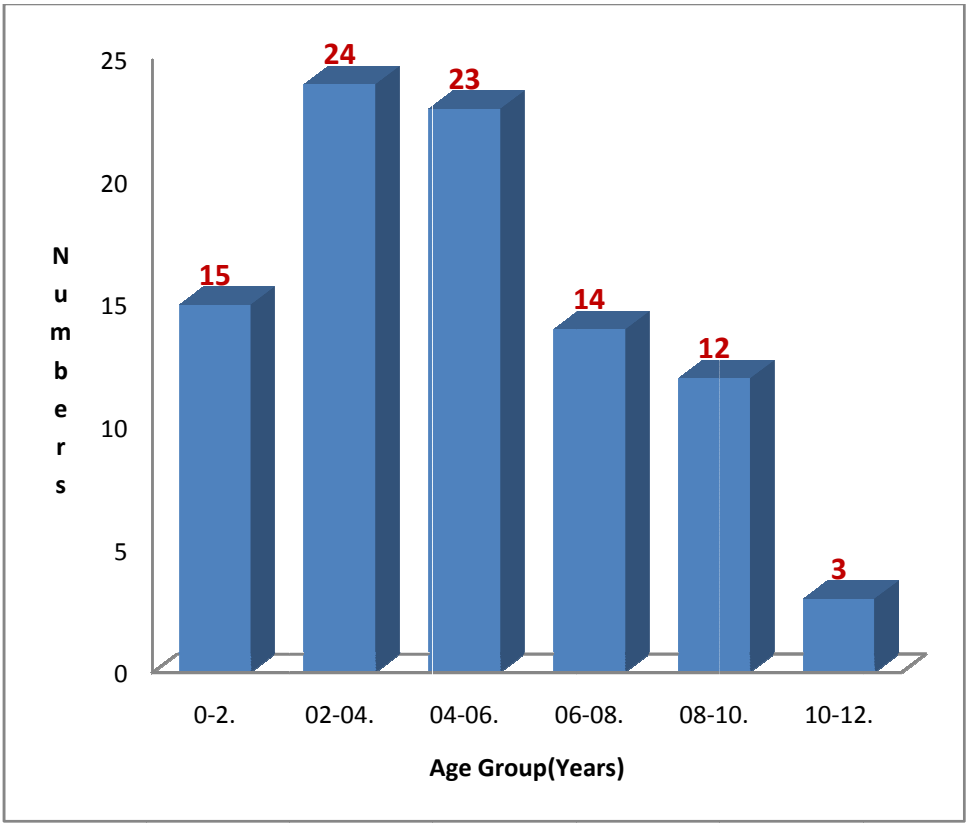


Figure 2- Age distribution

The above figure shows maximum cases reported were in between 2-6 years.

Table 1 – Age and gender distribution

		Gender		Total
		Male	**Female**	
Age Group	**0-02**	8	7	15
	02-04	16	8	24
	04-06	18	5	23
	06-08	7	7	14
	08-10	5	7	12
	10-12	3	0	3
Total		57	34	91

Table 2

Clinical Features

	Present (%)	Absent (%)	Total
Head ache	69(75.8)	22(24.2)	91(100)
Fever	91(100)	0(0)	91(100)
Temp >39C	91(100)	0(0)	91(100)
Conjunctival Suffusion	13(14)	78(86)	91(100)
Meningism	1(99)	90(1)	91(100)
Muscle pain	76(84)	15(16)	91(100)
Conjunctival Suffusion and Meningism	7(8)	84(92)	91(100)
Jaundice	17(19)	74(81)	91(100)
Albuminuria/Nitrogen Retention	3(3)	88(97)	91(100)
Rainfall	25(28)	66(72)	91(100)
Contact with Cont.Environment	79(87)	12(13)	91(100)
Animal Contact	53(58)	38(42)	91(100)

All had clinical Fever/Temp >39®c followed by headache and myalgia in descending order .

Epidemiological features:

Table 3- Rainfall

Feature	Number	percentage
Rain fall	25	28
No Rain fall	66	72

Table 4- contact with contaminated environment

Contact with Cont.Environment	Number	percentage
Present	79	87
Absent	12	13

Table 5- Animal contact

Animal Contact	Number	percentage
Present	53	58
Absent	38	42

Serology :

MSAT : 27(30%) were sero positive with MSAT.

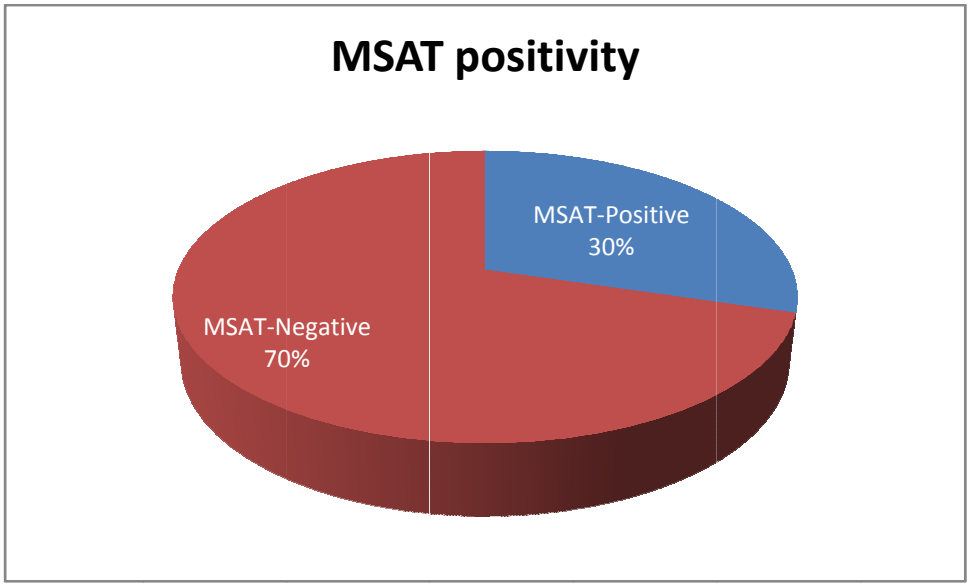


Figure 3

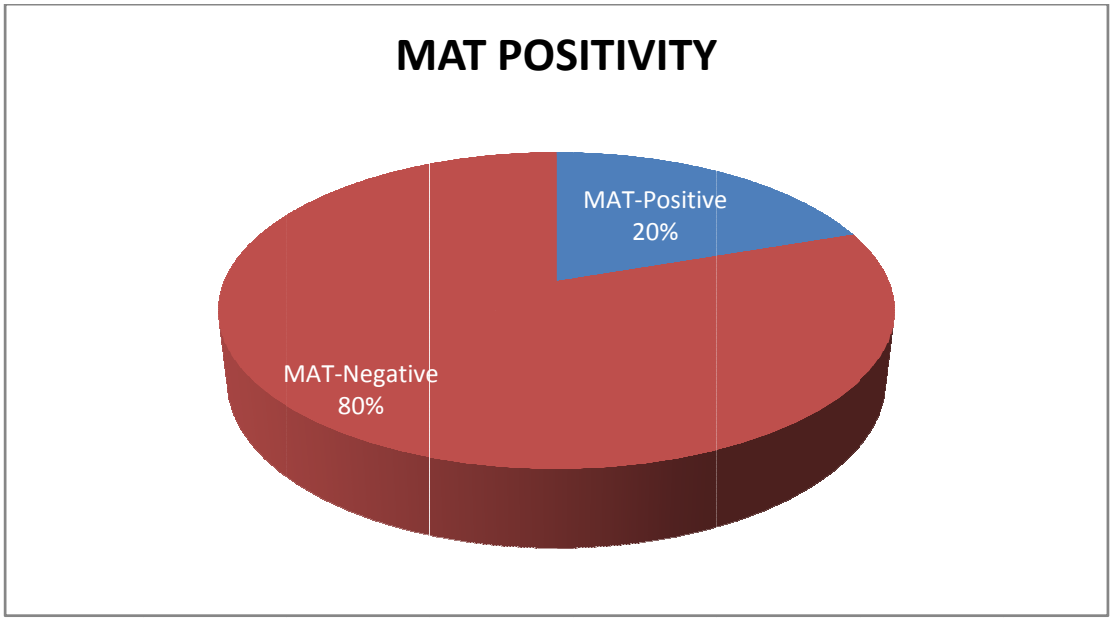


Figure 4

In our study MAT positivity was 20% (18 cases).

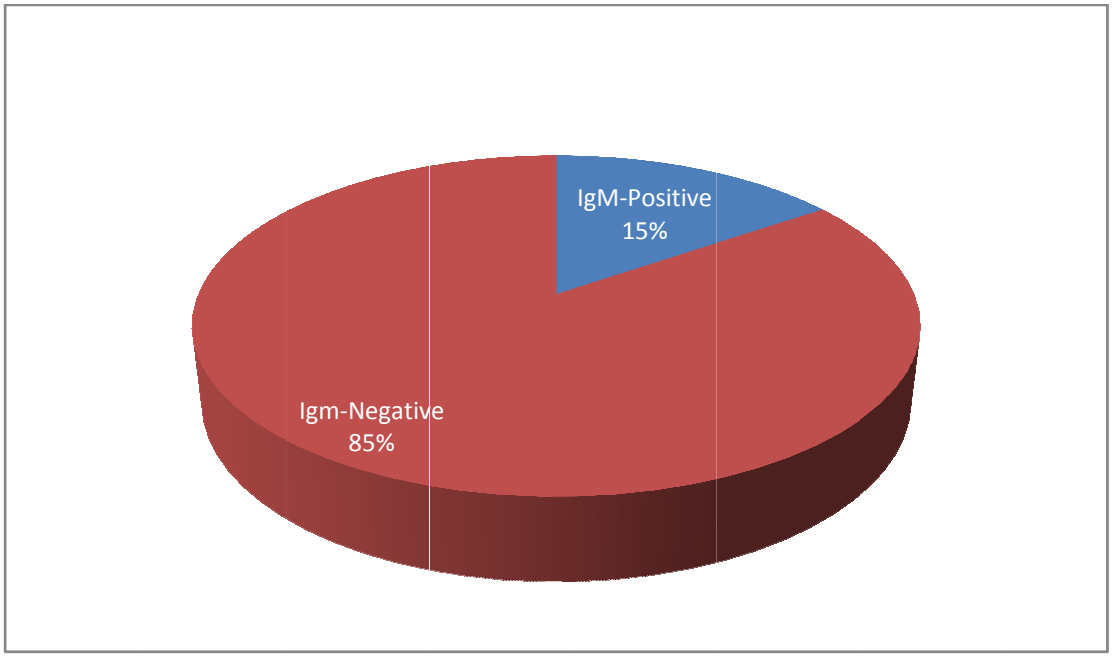


Figure 5- IgM Elisa

The above diagram depicts the positivity.

IgM Elisa in diagnosed patients with Leptospirosis

Table 6- IgM Elisa

IgM-Positive	14 cases
Igm-Negative	77 cases

Table 7

Effectiveness of IgM Elisa test:

		MAT		
		Positive	Negative	Total
IgM	Positive	6	8	14
	Negative	12	65	77
	Total	18	73	91

Table 8

	ESTIMATE	**95 % CI**
Sensitivity	33.3	15.6-52.3
Specificity	89.0	84.7-93.7
Efficacy	78	71.0-85.5
Positive Predictive Value	42.5	20.0-67.2
Negative Predictive Value	84.4	80.3-88.8
False Positivity Rate	11.0	5.2-14.12
False Negativity Rate	66.7	59.63-77.89
Likelihood Ratio +	3.042	1.015-8.303
Likelihood Ratio -	0.749	0.510-0.997
Cohens Kappa test	0.224	Fair agreement
McNemar TEST	0.503	Not Significant

Table 7 and 8 shows shows the sensitivity, specificity, efficacy,positivepredictive value, negative predictive value , false positivity rate, false negativity rate , likelihood ratio +, likelihood ratio -, cohen's kappa test and Mc'Nemar Test with 95 % confidence interval for IgM elisa.

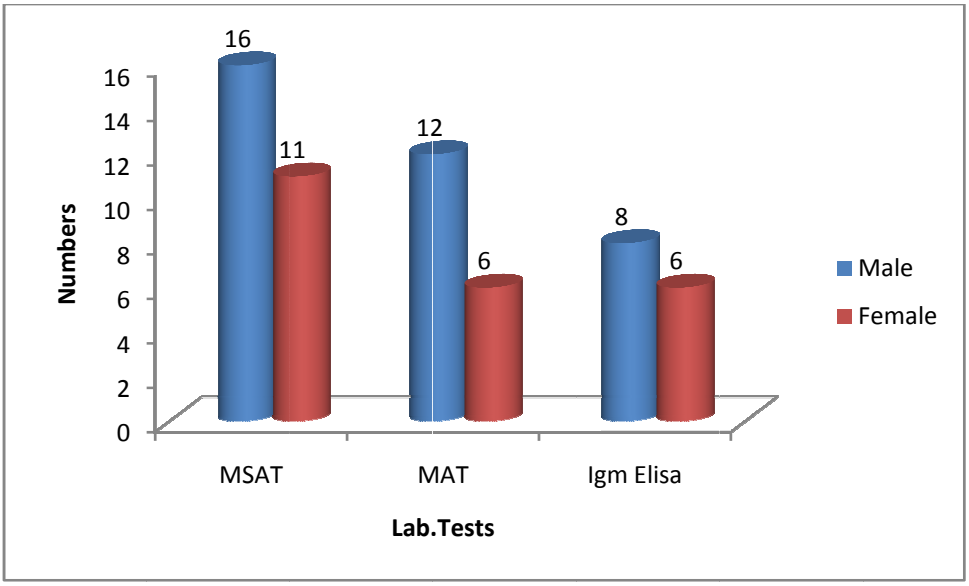


Figure 6

The above diagram depicts the positivity of MAT MSAT and IgM Elisa in diagnosed patients with Leptospirosis

LEPTOSPIROSIS POSITIVITY:

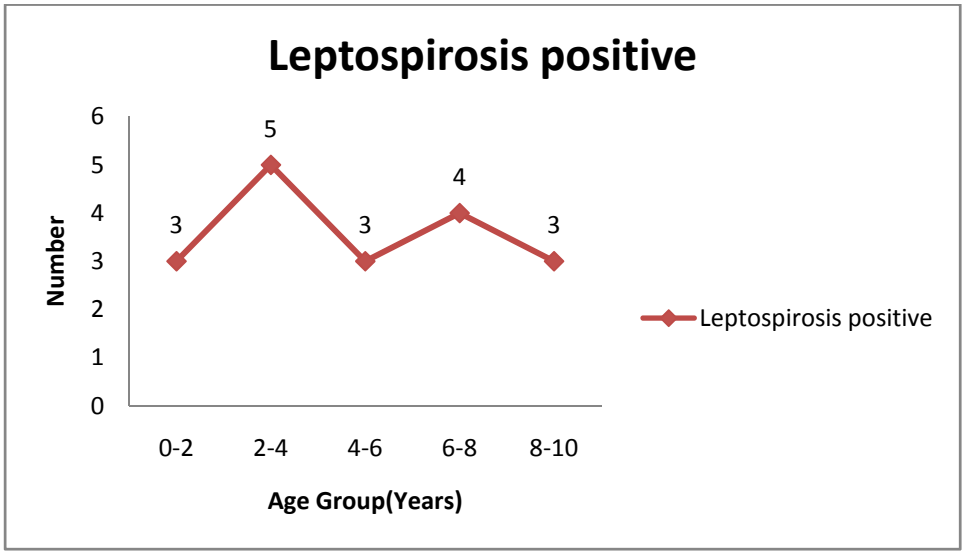


Figure 7

The above figure shows distribution of MAT positivity with Positive Letospirosis in 18 cases with positivity rate of 19.78%.

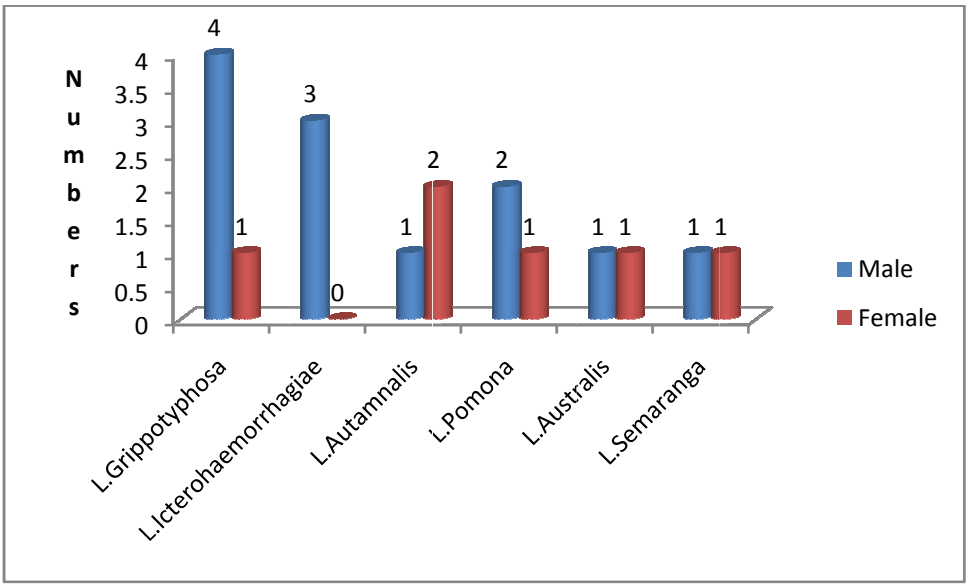


Figure 8

The above table and diagram shows the type of serovars detected by MAT. Among various serovars L.grippotyphosa occurred most commonly followed by icterohaemorrhagiae, L.autumnalis,L.pomona,L.australis,L.semarana.

Faine's and modified faine's criteria:

Table 9

	Faines (%)	Modified Faines (%)
Letospirosis positive	23(25)	39(43)
Letospirosis negative	68(75)	52(57)
Total	91(100)	91(100)

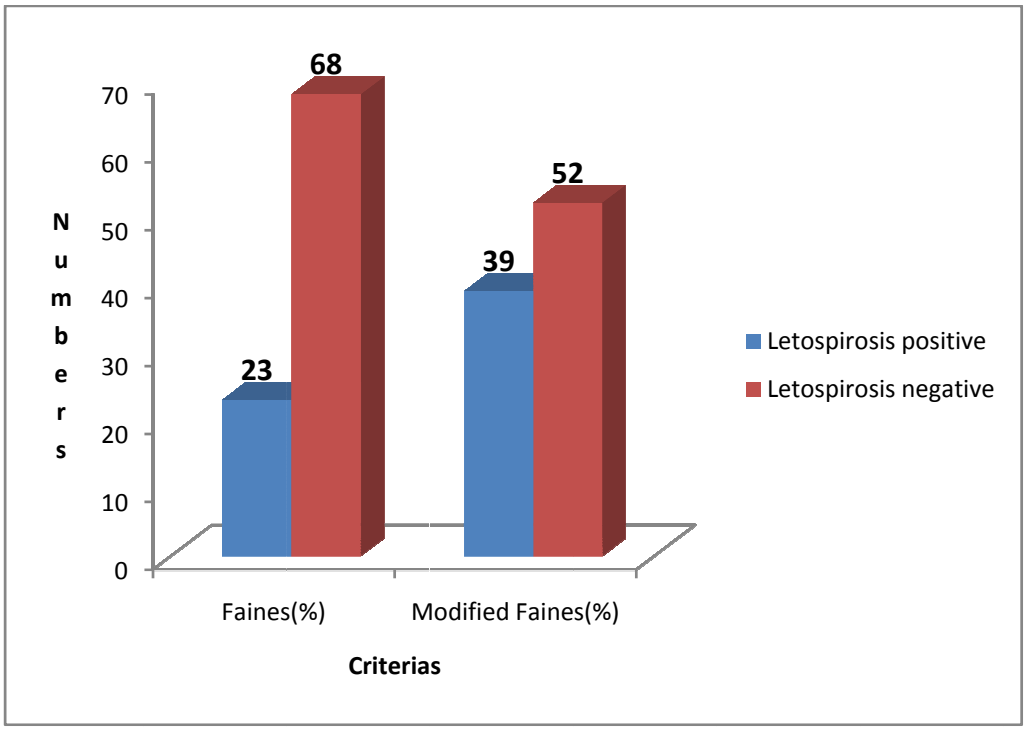


Figure 9

The above table and figure shows the distribution of faine's and modified faine's criteria.

Faines criteria:

Table 10

		MAT		
		Positive	Negative	Total
Faines Criteria	Positive	2	21	23
	Negative	16	52	68
	Total	18	73	91

Table 11

	ESTIMATE	**95 % CI**
Sensitivity	11.1	2-32
Specificity	71.2	69-76
Efficacy	59.3	55.7-68
Positive Predictive Value	8.7	1.5-25
Negative Predictive Value	76.5	74.1-82.2
False Positivity Rate	28.76	17.22-30.12
False Negativity Rate	88.88	77.23-89.89
Likelihood Ratio +	0.386	0.064-1.406
Likelihood Ratio -	1.248	0.876-1.421
Cohens Kappa test	-0.160	slight agreement
McNemar TEST	0.511	NS

Modified Faines criteria :

Table 12

		MAT		
		Positive	Negative	Total
Modified Faines Criteria	Positive	17	22	39
	Negative	1	51	52
	Total	18	73	91

Table 13

	ESTIMATE	**95 % CI**
Sensitivity	94.4	73-99.7
Specificity	69.9	64.6-0.712
Efficacy	74.7	66.3-76.8
Positive Predictive Value	43.6	33.7-46
Negative Predictive Value	98.1	90.7-99.9
False Positivity Rate	30.13	18.3-31.4
False Negativity Rate	5.55	3.45-6.01
Likelihood Ratio +	3.314	2.063-3.457
Likelihood Ratio -	0.080	0.004-0.417
Cohens Kappa test	0.447	Moderate Agreement
McNemar TEST	P<0.001	Highly significant

Table 11 and 13 shows shows the sensitivity, specificity, efficacy , positive predictive value, negative predictive value , false positivity rate , false negativity rate , likelihood ratio +, likelihood ratio -, cohen's kappa test and Mc'Nemar Test with 95 % confidence interval for Faine's and modified Faine's criteria.

DISCUSSION

In our study of 91 children, 14(15%) of them were diagnosed to have Leptospirosis on the basis of positive IgM ELISA test. Sunil Karande S et al in Mumbai has reported a diagnosis of leptospirosis in 30 out of 93 (32%) children who were suspected to have the same.[23] This increase in positivity of Mumbai study might have been because these cases were evaluated in children living in slums and immediately following floods.

Clinical diagnosis of leptospirosis is difficult, hence a high index of suspicion is required for the diagnosis when a patient presents with fever, headache, and myalgia.[81] Similar observations in the differential diagnosis of Leptopsirosis have been made in earlier studies. [81, 82]

AGE GROUP

Majority of cases with leptospirosis in our series were between 2-6 years of age (51.6%) which was similar to karande S et al.

GENDER DISTRIBUTION

Male: female ratio in our series was 1.7:1. It was reported to be 1.6:1 by karande S et al.[23] There was a similar male preponderance 88 (63%) in the study by Rajajee S et al.[82] In adults the disease is 5 times

more common in males, probably because of the occupation and activity.[86]

TYPE OF WATER SUPPLY

Nearly one fourth of the families of children with leptospirosis did not use protected water supply for drinking. Protected water supply can prevent the disease.[29, 30] This finding implies the need for providing protected water supply to all which is very difficult in a vast country like ours.

DURATION OF HOSPITAL STAY

The duration of hospital stay was < 5 days in 50.54 %(46), between 5 – 10 days in 37.6% (34) and between 11-15 days in 12.08%(11) children in our study. This was comparable to the mean symptom duration of 10.1 days as reported by karande S el al .[23]

CLINICAL FEATURES

Headache (75.8%) and myalgia (84%) were the most common symptoms next to fever. Headache was reported in 54% of cases in a study by karande S et al, the higher occurrence of headache might have been, because their series had more children in the older age group.[23] The occurrence of myalgia was similar to the observation from an earlier study at Chennai by Rajajee S et al which reported 24 % .[82] Conjunctival suffusion is present in 21.9 % in our series, 15% in series of both

karande et al and Rajajee S et al. [23,82] Meningeal irritation was present in 8.79% patient in our study, similar to the observation in study by Rajajee S et al(7%) and meningismus was not observed in study by karande S et al.[23,82] Less number of children in our series had jaundice (18.68 %) when compared to earlier reports from Mumbai (36 %) and comparable to reports from Chennai (18 %) . [23, 82] In adults approximately 10% of those infected become jaundiced.

Outcome

All patients recovered well in our study. None of our patients had renal failure. This was similar to two studies by karande S et al where the illness was relatively mild and anicteric in all the cases and the patients did not have any complications.[3, 23]

MAT Seropositivity

A total of 18(20%) out of 91 children in the series tested positive by MAT. A Serosurvey undertaken by Ratnam et al, among conservancy workers in Chennai using MAT found a seropositive prevalence rate ranging between 17.8% to 40.5%.[93] Swapna et al from Calicut in Kerala has shown the seroprevalence to be 38.1% in the high-risk group vs. 24% in healthy controls like students and blood donors. [94] This necessitates the need for caution in the interpretation of MAT in endemic areas.

IgM ELISA

IgM ELISA was positive in 14 patients (15%). IgM ELISA was performed during 5 days to 2 week in 69 patients (75.82%) and more than 2 weeks in 22 patients (24.7%). Sensitivity and specificity of IgM Elisa by comparing with MAT in our study were 33.3% and 89% respectively.

MSAT

MSAT was positive in 27 patients (30%). A study of 108 cases of leptospirosis from Brazil has revealed that 65% of the first sample positive by MSAT when compared to 44% by MAT.[45] This low positivity could be because of difficulty in reading by dark field microscope.

Faine's criteria and modified Faine's criteria

The sensitivity of 11.1 % and specificity of 71.2% observed in our series for Faine's Criteria is less than that reported by Rao et al in their earlier report (88.9%, 80.2%). [73] Our study was conducted only in children and it is possible that some of the children might not have complained of headache and myalgia like adults .

Lower sensitivity observed in our series for Faine's criteria could have been because our study was conducted in an endemic area in contrast to the study by Rao et al, which was done in a non – endemic area.

The sensitivity and specificity (94.4%, 69.9%) for modified Faines criteria in our series was similar to the observations in an earlier study in adults by Shivakumar et al. [81] The reason why modified Faine's criteria had higher sensitivity and specificity is probably because IgM Elisa and MSAT has been used in making the diagnosis of Leptospirosis.

Modified Faine's criteria had high sensitivity and specificity compared to Faine's criteria in our series, this suggests that modified Faine's criteria might be more useful in the diagnosis of leptospirosis. Very similar observations have been made by Shivakumar et al in the only available study on modified Faine's criteria in literature.

Following are the few examples utilizing modified Faine's criteria

EXAMPLES UTILISING MODIFIED FAINE'S CRITERIA

Patient with fever during the monsoon month with positive ELISA IgM

Score	A	Fever	2
	B	Rain Fall + Contact with contaminated environment	9
	C	ELISA Ig M positive	15
Score		= 2+9+15 = 26	
Diagnosis		Leptospirosis (confirmed)	

It should be realized that clinical data on milder anicteric forms of Letospirosis in inadequate in our country and this can be made only if simple tests are done in small laboratories.

Limitations of this study

1. Cultures which are the gold standard were not performed.

2. As per WHO criteria only presumptive diagnosis of Leptospirosis was made.

3. Cases in our series were studied only after the five days of the illness.

SUMMARY

This descriptive study for a period of two years was conducted in the Institute of child health & hospital for children, Egmore, Chennai. Children from age group of 1 year to 12 years with fever for more than 5 days with signs and symptoms suggestive of leptospirosis were included in the study.

Aim of the study was to evaluate Faine's, modified Faine's criteria and IgM Elisa in the diagnosis of leptospirosis.

In our study out of 91 children 14 were diagnosed as leptospirosis using IgM ELISA, 23 and 39 were diagnosed as leptospirosis using Faine's and modified Faine's criteria respectively. The results were compared with MAT.

Modified Faine's criteria had high sensitivity and specificity compared to Faine's criteria in our series, this suggests that modified Faine's criteria might be more useful in the diagnosis of leptospirosis.

CONCLUSION

1. Diagnosis of Leptospirosis was made by positive Leptospira IgM ELISA test in nearly 15.38% of children with suspected leptospirosis.
2. Leptospirosis infection may masquerade as multiplicity of separate diseases whose clinical features vary considerably.
3. Headache and myalgia were the predominant symptoms in addition to fever.
4. The sensitivity & specificity of IgM Elisa were 33.3 % and 89 % respectively.
5. The sensitivity & specificity of modified Faine's criteria were 94.4 % and 69.9 % respectively which was significantly higher than Faines criteria
6. The sensitivity & specificity of Faine's criteria were 11.1 % & 71.2 % respectively.
7. All the three serological tests (MAT, MSAT & IgM ELISA) were positive only in about 3.29% of children.
8. The occurrence of cases was higher during the monsoon.
9. There was no mortality in our series of children with Leptospirosis.

RECOMMENDATIONS

Based on our observation that Modified Faine's criteria had better sensitivity and specificity than Faine's Criteria, the following recommendation is made.

Modified Faine's criteria might be utilized as a diagnostic test for leptospirosis, particularly in areas of high prevalence, so that specific antibiotic treatment might benefit children with leptospirosis.

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ANNEXURE - I
PROFORMA

Name	Serial no.
Age	IP no
Sex	DOA
Weight	DOD
Address	DOB
Monthly income	UNIT
State	
District	
Occupation of Father & Mother	
Contact no	
Socio economic group	
Day of fever on the day of testing	Fever settled on

Symptoms

Fever	Y/N	Max recorded temperature
Headache	Y/N	
Myalgia	Y/N	
Joint pain	Y/N	
Vomiting	Y/N	
Jaundice	Y/N	
Abdominal pain	Y/N	
Loose stools	Y/N	
Edema	Y/N	
Seizures	Y/N	

Bleeding Epistaxis Hematemesis Melena

Oliguria Anuria Hematuria

Others

Source of water supply protected/unprotected

Animals at home cats dogs cows rats

Drugs before admission

General Examination

HR RR BP Anemia Transfused y/n

Icterus Edema Rashes Temp. Lymphadenopathy

Facial puffiness Muscle tenderness conjunctival suffusion

Systemic Examination

CVS

RS

CNS

P/A

INVESTIGATIONS

MAT - ACUTE CONVALESCENT

MSAT - ACUTE CONVALESCENT

ELISA Ig M

Hb PCV

TC DC

Total Bilirubin Platelet Count

ESR Blood Culture

AST/ALT Serum Proteins/Serum Albumin

PT PTT

Serum Creatinine Blood Urea

CRP CPK/MB

Urine Examination Urine Culture

USG Abdomen CSF Analysis

ECHO ECG

Treatment given

Outcome Death Improved Discharged Worsened
Dialysis Ventilation ICU stay

Final diagnosis

Reason for exclusion

Faine's Criteria		**Modified Faine's Criteria**	
Part A : Clinical Data		**Part A : Clinical Data**	
Question	**Score**	**Question**	**Score**
Headache	2	Headache	2
Fever	2	Fever	2
Temp > 39ºC	2	Temp > 39ºC	2
Conjunctival suffusion	4	Conjunctival suffusion	4
Meningism	4	Meningism	4
Muscle pain	4	Muscle pain	4
Conjunctival suffusion + Meningism + Muscle pain	10	Conjunctival suffusion + Meningism + Muscle pain	10
Jaundice	1	Jaundice	1
Albuminuria/Nitrogen Retention	2	Albuminuria/Nitrogen Retention	2
Total score		Total score	

Part B: Epidemiological factors		**Part B: Epidemiological Factors**	
Contact with animals or Contact with known Contaminated water	**10**	Rainfall	**5**
		Contact with contaminated environment	**4**
		Animal contact	**1**
total		total	

Part C: Bacteriological and Lab Findings		**Part C: Bacteriological and Lab Findings**	
Isolation of leptospira in culture – Diagnosis certain		Isolation of leptospira in culture – Diagnosis certain	
Positive Serology (MAT)		**Positive Serology**	
Leptospirosis Endemic			
Single positive Low titre	2	ELISA IgM Positive *	15
Single positive– High titre	10	SAT – Positive *	15
		MAT – Single High titer*	15
Leptospirosis Non Endemic			
Single positive – Low titre	5		
Single positive– High titre	15	Rising titer (Paired sera)	25
Rising titre (Paired sera)	25		

- Any one of the tests only should be scored

ANNEXURE II
Patient Information Sheet

Aim of the study

To study the Evaluation of faine's criteria and IgM ELISA in the diagnosis of leptospirosis.

Among children admitted in a tertiary care hospital.

All datas will be kept strictly private and confidential you may choose to take part or not in this study. That is your choice no penalties or loss of benefit will come from refusing. If you chosen to take part, you may refuse to answer any question.

If you have any doubts regarding you can meet the investigator. In this study all investigations are done at free of cost. The treatment given also free of cost.

SECTION - 2

Informed Consent Form

I agree to participate in the study titled Evaluation of faine's criteria and IgM ELISA in the diagnosis of leptospirosis.

I confirm that I have been told about this study in my mother tongue (Tamil) and I had the opportunity to ask questions. I confirm that I have been told about the risks and potential benefits being affected.

I agree not to restrict the use of any data or results that arise from this study.

Name of the child :
Signature :
Date :

Name of Guardian / care giver :
Signature :
Date :

Name of the witness :
Signature :
Date :

Name of investigator :
Signature :
Date :

தகவல் தாள்

ஆய்வின் நோக்கம்:

குழந்தைகளுக்கு எலிக் காய்ச்சல் (Leptospirosis) வருவதை கண்டுபிடிப்பதற்கான வழிகளையும், அதன் உறுதி தன்மையையும் அறிவதே இந்த ஆய்வின் நோக்கம்.

சட்டத்தினால் வழங்கப்பட்டபடி சேகரிக்கப்பட்ட விவரம் இரகசியமாக பாதுகாக்கப்படும். இந்த ஆய்வில் தங்கள் குழந்தை பங்கேற்பது உங்களது விருப்பத்தை பொறுத்தது. இந்த ஆய்வில் இருந்து விலகுவதால் மருத்துவ சிகிச்சை அளிப்பதில் எந்தவித இடறும் நேராது. ஆய்வில் பங்கேற்கும்போது இடையில் விலகவோ, கேள்விகளுக்கு விடையளிக்காமல் இருக்கவோ தங்களுக்கு உரிமை உள்ளது.

ஆய்வு குறித்து தங்களுக்கு ஏதேனும் சந்தேகம் ஏற்பட்டால் ஆய்வாளரை நேரில் சந்திக்கவோ, தொலைபேசியில் தொடர்பு கொள்ளவோ வரவேற்கப்படுகிறீர்கள்.

அபாயங்கள் மற்றும் நன்மைகள்:

இந்த ஆய்வில் பங்கேற்பதால் எந்தவித தீங்கும் ஏற்பட வாய்ப்பில்லை இதில் பங்கேற்கும் போது செய்யப்படும் மருத்துவ பரிசோதனைகள் மற்றும் சிகிச்சை முற்றிலும் இலவசமானது.

ஒப்புதல்:

இந்த தொடராய்வில் செய்யப்படுகின்ற செய்முறைகளால் ஏற்படும் பக்க விளைவுகளுக்கு மருத்துவ உதவி செய்யப்படும். எந்தவித நஷ்ட ஈடும் தரப்படமாட்டாது என்பதையும் அறிந்து கொண்டேன்.

1. நான் இந்த ..... தேதியிட்ட தகவல் படிவத்தை நன்றாக படித்து காட்டி எடுத்துரைத்ததை புரிந்துகொண்டேன். எனக்கு கேள்வி கேட்கும் வாய்ப்பு கிடைத்தது.

2. இந்த ஆய்வில் நான் என்னுடைய சுய அறிவோடு பங்கு கொள்கிறேன். மேலும் இந்த ஆய்விலிருந்து எந்தவிதக் காரணமும் தராமல் மருத்துவப் பரிசோதனையிலிருந்து நான் விலகிக் கொள்ளலாம். இதனால் சட்டரீதியான எந்த செயலும் உட்படுத்தாது.

3. Ethics குழுவின் அங்கத்தினர்களோ, இந்த ஆய்வினை நடத்துபவர்களோ என்னுடைய மருத்துவ ஆய்வின் அனைத்து விவரங்களையும் என்னுடைய அனுமதியின்றி பார்க்கவோ, படிக்கவோ உரிமையுள்ளவர்களாவர். நான் இந்த ஆய்விலிருந்து விலகிக் கொண்டாலும்கூட என்னுடைய விவரங்களை அவர்கள் அறிந்துகொள்ள ஒத்துக்கொள்கிறேன். என்னுடைய விவரங்கள் அனைத்தும் 3-வது நபருக்கோ அல்லது பத்திரிகைகளில் வெளியிடுவதற்கோ முயலமாட்டீர்கள் என நம்புகிறேன்.

4. இந்த ஆய்விலிருந்து பெறப்பட்ட புள்ளி விவரங்களையோ அல்லது முடிவுகளையோ பயன்படுத்தகூடாது என்று கட்டுப்படுத்த மாட்டேன்.

5. என் குழந்தையை இந்த மருத்துவ ஆய்விற்கு பங்கு கொள்ள பரிபூரணமாக சம்மதிக்கிறேன்.

கையொப்பம்..... தே.....

பெயர் .....

குழந்தையின் பெயர் .....

Signature of the Investigator : Date :

Signature of the Witnesses : Date :

ANNEXURE III

ABBREVIATIONS

MAT	-	microscopic agglutination test
ELISA	-	Enzyme-linked immunosorbent assay
MSAT	-	Macroscopic slide agglutination tests
PCR	-	polymerase chain reaction
DFM	-	Dark-field Microscopy
CDC	-	centers for disease control and prevention.
WHO	-	World health organisation
CI	-	Confidence interval
ALT	-	Alanine aminotransferase(serum glutamate pyruvate transaminase)
AST	-	aspartate aminotransferase (serum glutamic-oxaloacetic transaminase)